

What is claimed is:

1. A binary transgenic viral expression system comprising:
  - (i) a chromosomally-integrated inactive replicon comprising:
    - a) *cis*-acting viral elements required for viral replication;
    - b) a target gene comprising at least one suitable regulatory sequence; and
    - c) site-specific sequences responsive to a site-specific recombinase; and
  - (ii) a chromosomally-integrated chimeric transactivating gene comprising a regulated plant promoter operably-linked to a site-specific recombinase coding sequence;

wherein expression of the chimeric transactivating gene in cells containing the inactive replicon results in the site-specific recombination, activation of replicon replication, and increased expression of the target gene.
2. The method of Claim 1 wherein the site-specific sequences responsive to the recombinase are lox sequences and the site-specific recombinase coding sequence encodes for the Cre protein.
3. The transgenic viral expression system of Claim 1 wherein the inactive replicon is derived from viruses selected from the group consisting of geminiviruses and single-stranded RNA viruses.
4. The transgenic viral expression system of Claim 3 wherein the geminivirus is selected from the group consisting of TGMV and ACMV.
5. The transgenic viral expression system of Claim 3 wherein the single-stranded RNA viruses is a potato virus X.
6. The viral expression system of Claim 1 wherein the regulated plant promoter is selected from the group consisting of tissue-specific promoters, inducible promoters, and development stage-specific promoters.
7. The viral expression system of Claim 6 wherein the regulated promoter is derived from genes selected from the group consisting of genes derived from a safener-inducible system, genes derived from the tetracycline-inducible system, genes derived from salicylate-inducible systems, genes derived from alcohol-inducible systems, genes derived from glucocorticoid-inducible system, gene derived from pathogen-inducible systems, and gene derived from ecdysome-inducible systems.
8. The viral expression system of Claim 1 wherein the target gene encodes a protein selected from the group consisting of an enzyme, a structural protein, a seed storage protein, a protein that conveys herbicide resistance, and a protein that conveys insect resistance.

9. The viral expression system of Claim 1 wherein the target gene encodes an RNA whose expression results in homology-dependent gene silencing of a transgene or endogenous gene.

10. The viral expression system of Claim 1 wherein the at least one suitable regulatory sequence linked to the target gene is selected from the group consisting of constitutive plant promoters, plant tissue-specific promoters, plant development-specific promoters, inducible plant promoters and viral promoters.

11. The viral expression system of Claim 10 wherein the at least one suitable regulatory sequence is selected from the group consisting of a viral coat protein promoter, the nopaline synthase promoter, the phaseolin promoter, and the cauliflower mosaic virus promoter.

12. The viral expression system of Claim 1 wherein the inactive replicon optionally contains a DNA fragment encoding a transit peptide.

13. A method of altering the levels of a protein encoded by a target gene in a plant comprising:

- (i) transforming a plant with the viral expression system of Claim 1; and
- (ii) growing the transformed plant seed under conditions wherein the protein is expressed.

14. The method of Claim 13 wherein the target gene is in sense orientation and the level of the expressed protein is increased.

15. The method of Claim 13 wherein the site-specific sequences responsive to the recombinase are mutant lox sequences that are inefficient for Cre-lox recombination and the site-specific recombinase coding sequence encodes for the Cre protein.

16. A method of altering the levels of a protein encoded by a target gene in a plant comprising:

- (i) transforming a first plant with a inactive replicon to form a first primary transformant, the inactive replicon comprising:
  - a) *cis*-acting viral elements required for viral replication;
  - b) a target gene comprising at least one suitable regulatory sequence; and
  - c) site-specific sequences responsive to a site-specific recombinase,
- (ii) transforming a second plant with a chimeric transactivating gene to form a second primary transformant comprising a regulated plant promoter operably-linked to a transactivating site-specific recombinase coding sequence;

- (iii) growing the first and second primary transformants wherein progeny from both seeds are obtained; and
- (iv) crossing the progeny of the first and second transformants wherein the target gene is expressed.

5           17. The binary transgenic expression system of Claim 1 wherein the chromosomally-integrated inactive replicon is inserted into a reporter gene sequence such that when the replicon is excised the reporter gene is activated.

          18. The binary transgenic expression system of Claim 1 wherein a Transcription Stop Fragment is inserted in the inactive replicon.

10           19. A binary transgenic viral expression system comprising:

- (i) a chromosomally-integrated inactive replicon comprising:
  - a) *cis*-acting viral elements required for viral replication;
  - b) a target gene comprising at least one suitable regulatory sequence; and
  - 15           c) site-specific sequences responsive to a site-specific recombinase; and
- (ii) a transiently-expressed chimeric transactivating gene comprising a plant or viral promoter operably-linked to a site-specific recombinase coding sequence;

20           wherein expression of the chimeric transactivating gene in cells containing the inactive replicon results in the site-specific recombination, activation of replicon replication, and increased expression of the target gene.

          20. A method of altering the levels of a protein encoded by a target gene in a plant comprising:

- 25           (i) transforming a plant with a inactive replicon the inactive replicon comprising:
  - a) *cis*-acting viral elements required for viral replication;
  - b) a target gene comprising at least one suitable regulatory sequence; and
  - 30           c) site-specific sequences responsive to a site-specific recombinase;
- (ii) infecting the transformant with a virus containing a chimeric transactivating gene comprising a regulated plant promoter operably-linked to a transactivating site-specific recombinase coding sequence;

35           wherein expression of the chimeric transactivating gene in cells containing the inactive replicon results in the site-specific recombination, activation of replicon replication, and increased expression of the target gene.

21. A binary transgenic expression system comprising an inactive transgene and a chimeric transactivating gene, the inactive transgene comprising:

- (i) *cis*-acting transcription regulatory elements inoperably-linked to the coding sequence or functional RNA, and
- 5 (ii) site-specific sequences responsive to a site specific recombinase;

the chimeric transactivating gene comprising a regulated plant promoter operably-linked to a transactivating site-specific recombinase coding sequence, wherein expression of the chimeric transactivating gene in cells containing the inactive  
10 transgene results in an operable linkage of *cis*-acting transcription regulatory elements to the coding sequence or functional RNA through the site-specific recombination and increased expression of the target gene.

22. The binary transgenic expression system of Claim 21 wherein the site-specific sequences responsive to the recombinase are lox sequences.

15 23. The viral expression system of Claim 21 wherein the lox sequences are mutant lox sequences that are inefficient for Cre-lox recombination.

24. A binary transgenic viral replication system comprising:

- (i) a chromosomally-integrated inactive replicon comprising  
20 *cis*-acting viral elements required for viral replication and site-specific sequences responsive to a site-specific recombinase; and
- (ii) a chimeric transactivating gene, comprising a regulated plant promoter operably-linked to a site-specific recombinase coding sequence;

25 wherein expression of the chimeric transactivating gene in cells containing the inactive replicon results in the site-specific recombination and activation of replicon replication.

25. The method of Claim 24 wherein the site-specific sequences responsive to the recombinase are lox sequences.

30 26. The transgenic viral replication system of Claim 24 wherein the inactive replicon is derived from viruses selected from the group consisting of geminiviruses and single-stranded RNA viruses.

27. The transgenic viral replication system of Claim 26 wherein the geminivirus is selected from the group consisting of TGMV and ACMV.

35 28. The transgenic viral replication system of Claim 26 wherein the single-stranded RNA viruses is a potato virus X.

29. The viral replication system of Claim 24 wherein the regulated plant promoter is selected from the group consisting of tissue-specific promoters, inducible promoters, and development stage-specific promoters.

5 30. The viral replication system of Claim 29 wherein the regulated promoter is derived from genes selected from the group consisting of genes derived from a safener-inducible system, genes derived from the tetracycline-inducible system, genes derived from salicylate-inducible systems, genes derived from alcohol-inducible systems, genes derived from glucocorticoid-inducible system, gene derived from pathogen-inducible systems, and gene derived from  
10 ecdysome-inducible systems.

31. A binary transgene expression system of Claim 21, wherein, the inactive transgene is a silencing suppressor gene.

32. A binary transgenic expression system comprising:

- 15 (i) a chromosomally integrated blocking fragment bounded by site-specific sequences responsive to a site-specific recombinase; and  
(ii) a chromosomally integrated inactive silencing suppresser transgene;

20 wherein expression of the site specific recombinase results in the site-specific recombination that activates the silencing suppressor gene.

33. The binary transgenic viral expression system of Claim 32 wherein the blocking fragment is an inactive replicon comprising:

- 25 (i) a target gene comprising at least one suitable regulatory sequence; and  
(ii) site-specific sequences responsive to a site-specific recombinase;

wherein expression of the site specific recombinase results in the site-specific recombination, and activation of both the replicon and the silencing suppressor gene, and the increased expression of the target gene.

30 34. The binary transgenic viral expression system of Claim 32 wherein the blocking fragment is an inactive replicon comprising site-specific sequences responsive to a site-specific recombinase, wherein expression of the site specific recombinase results in the site-specific recombination, and activation of both the replicon and the silencing suppressor gene.

35 35. The binary transgenic viral expression system of Claim 32 wherein the silencing suppresser gene is selected from the group consisting of genes encoding PI-HC-Pro, HC-Pro, and 2b protein.

36. The binary transgenic viral expression system of Claim 32 wherein the silencing suppresser gene is selected from the group consisting of genes encoding BL1 or BR1 geminivirus movement proteins.

37. A transgenic viral expression system comprising:

- 5 (i) a chromosomally-integrated geminivirus proreplicon comprising:
- a) cis-acting viral elements required for viral replication;
  - b) a target gene comprising at least one suitable regulatory sequence; and
  - 10 c) flanking sequences that enable the excision of the elements of a) and b),

wherein the proreplicon lacks a functional replication gene for episomal replication;

- 15 (ii) a chromosomally-integrated chimeric *trans*-acting replication gene comprising a regulated plant promoter operably-linked to a geminivirus viral replication protein coding sequence; and

- (iii) a dimer of the geminivirus B genome;

20 wherein expression of the *trans*-acting replication gene in cells containing the proreplicon results in the replication of the proreplicon and the B-genome, and increased expression of the target gene.

38. A method of altering the levels of a protein encoded by a target gene in a plant comprising:

- 25 (i) transforming a plant with the viral expression system of Claim 37; and
- (ii) growing the transformed plant seed under conditions wherein the protein is expressed.

39. A transgenic geminivirus expression system comprising:

- 30 (i) a chromosomally-integrated inactive replicon comprising:
- a) *cis*-acting viral elements required for viral replication;
  - b) a target gene comprising at least one suitable regulatory sequence; and
  - c) site-specific sequences responsive to a site-specific recombinase;
- 35 (ii) a chromosomally-integrated chimeric transactivating gene comprising a regulated plant promoter operably-linked to a site-specific recombinase coding sequence;
- (iii) a dimer of a geminivirus B genome;

wherein expression of the chimeric transactivating gene in cells containing the inactive replicon results in the site-specific recombination, activation of replicon and B-genome replication, and increased expression of the target gene.

- 5       40. A method of altering the levels of a protein encoded by a target gene in a plant comprising:
- (i) transforming a plant with the viral expression system of Claim 39; and
  - (ii) growing the transformed plant seed under conditions wherein the protein is expressed.
- 10       41. A method of increasing viral resistance in a plant comprising:
- (i) transforming a first plant with a inactive replicon to form a first primary transformant, the inactive replicon comprising:
    - 15           a) *cis*-acting viral elements required for viral replication;
    - b) viral sequences homologous to the infecting virus capable of conferring homology-dependent resistance;
    - c) site-specific sequences responsive to a site-specific recombinase;
  - (ii) transforming a second plant with a chimeric transactivating gene to form a second primary transformant comprising a regulated plant promoter operably-linked to a transactivating site-specific recombinase coding sequence;
  - (iii) growing the first and second primary transformants wherein progeny from both seeds are obtained; and
  - (iv) crossing the progeny of the first and second transformants  
25           wherein the viral sequences homologous to the infecting virus are expressed, conveying viral resistance to the plant.
42. A binary transgenic viral expression system for replicating and increasing expression of a target gene comprising:
- 30       a) a heritable, chromosomally-integrated proreplicon lacking a functional replication gene for autonomous episomal replication, and comprising:
    - i) *cis*-acting viral elements required for viral replication;
    - ii) a target gene comprising at least one suitable regulatory sequence; and
    - 35           iii) flanking sequences that enable the excision of the elements of i) and ii); and

- b) a heritable, chromosomally-integrated chimeric *trans*-acting replication gene comprising a regulated plant promoter operably-linked to a viral replication protein coding sequence.
43. A ternary expression system comprising:
- 5 a) a first recombinase element comprising a first promoter operably linked to a sequence encoding a first recombinase;
- b) a second recombinase element comprising a second promoter, a stop fragment bounded by site specific sequences responsive to the first recombinase and a sequence encoding a
- 10 second recombinase wherein the presence of the stop fragment inhibits expression of the second recombinase, and wherein the first and second recombinases are different; and
- c) a DNA molecule bounded by site specific sequences responsive to the second recombinase;
- 15 wherein expression of the first recombinase excises the stop fragment from the second recombinase element, operably linking the second promoter and the sequence encoding the second recombinase, and wherein expression of the second recombinase results in site specific recombination within the DNA molecule bounded by site specific sequences responsive to the second recombinase.
- 20 44. The expression system of Claim 43 wherein the DNA molecule is a stop fragment.
45. The expression system of Claim 43 wherein the DNA molecule is a transgene comprising a third promoter operably linked to coding sequence.
46. The expression system of Claim 43 wherein the first, and second and
- 25 promoters are independently regulated.
47. The expression system of Claim 46 wherein the first, second and third promoters are independently regulated.
48. The expression system of Claim 45 wherein the transgene expression inhibited by the optional presence of a stop fragment, the stop fragment bounded
- 30 by site specific sequences responsive to the first recombinase.
49. A binary transgenic viral expression system for replicating and increasing expression of a target gene comprising
- a) a heritable, chromosomally-integrated proreplicon lacking a functional replication gene for autonomous episomal
- 35 replication, and comprising:



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- i) *cis*-acting viral elements required for viral replication;
  - ii) a target gene comprising at least one suitable regulatory sequence; and
  - iii) flanking sequences that enable the excision of the elements of i) and ii); and
- 10
- b) a heritable, chromosomally-integrated chimeric *trans*-acting replication gene comprising a regulated plant promoter operably-linked to a viral replication protein coding sequence.

50. The binary transgenic viral expression system of Claim 49 wherein the prereplicon and the *trans*-acting replication gene are independently derived from any geminivirus.

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51. The binary transgenic viral expression system of Claim 50 wherein the geminivirus is selected from the group consisting of TGMV and ACMV.